وزارة التعليم العالي والبحث العلمي
الجامعة التقنية الجنوبية
المعهد التقني العمارة
قسم تقنيات المختبرات الطبية

الحقيبة التدريسية لمادة تقنيات مختبرية عملي

الصف: الأول

تدريسي المادة

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الفصل الدراسي الأول

جدول مفردات مادة تقنيات مختبرية عملي

Week	Details
1-3	Introduction on the subject of medical laboratory techniques. - Glassware and materials used in some tests. Disinfection and sterilization (Chemical and physical) - biological and chemical hazards and safety
4	Samples collection and handling. - Samples collection for different lab. Investigations, samples transport, samples preparation.
5	Culturing of microorganism :- types of Culture media, preparation of culture media
6	Urine samples: Chemical and physical investigations, microscopic examination. Culture and sensitivity
7	Stool sample: General examination. Culture and sensitivity
8	Seminal Fluid: Seminal fluid examination Liquification time, physical examination, microscopic examination. Fructose test.
9	Heamagglutination test
10	Advance techniques -Enzyme-linked immunosorbent assay (ELISA) procedure, troubleshoot. Cutoff value, standard curve
11	Radioimmunoassay (RIA) procedure, troubleshoot.
12	Immunofluoresence technique
13	Polymerase chain reaction (PCR), types procedure, gel electrophoresis
14	Real-time PCR, procedure application in medical lab.
15	Review

الهدف من دراسة مادة تقنيات مختبرية (الهدف العام):

تهدف دراسة مادة تقنيات مختبرية للصف الأول إلى:

1- التعرف على المفاهيم العامة للمختبر الطبي.

2- التعرف على التقنيات الضرورية للعمل في المختبر الطبي.

الفئة المستهدفة: طلبة الصف الأول/قسم تقنيات المختبرات الطبية.

التقنيات التربوية المستخدمة:

1- سبورة وأقلام.

2- السبورة التفاعلية.

3- عارض شاشة Data Show.

4- جهاز حاسوب محمول Laptop.

الأسبوع الأول-الأسبوع الثالث

الهدف التعليمي:

التعرف على تقنيات المختبرات الطبية وكذلك التعرف على الأدوات الزجاجية الموجودة في المختبر الطبي والتعرف على بعض التقنيات المستخدمة في المختبرات الطبية مثل التعقيم والتطهير والتعرف على السلامة المختبرية وأنواع المخـــاطر في المختبر.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Laboratory Techniques:**

Introduction:

Laboratory techniques can be defined as the branch of laboratory medicine which deals with the examination of tissues and excretions of the human body and body fluids by various electronic, chemical, microscopic and other medical laboratory procedures or techniques either manual or automated which will aid the technician in the diagnosis, study and treatment of disease and in the promotion of health in general.

Clinical laboratory science professionals (also called medical laboratory scientists or medical laboratory technicians), are highly skilled scientists who determine the best treatment for the patient.

Laboratory Glassware:

Glassware are used in lab to contain or deliver liquids, usually manufactured from boro-silicate glass (Pyrex), is material with the following defined characteristics: resistant to the action of chemical and made to withstand sudden change of temperature. Such as cylinders, flasks, beakers, pipettes are graduated to certain volume, test tubes and funnels.

Sterilization and Disinfection:

1. **Sterilization:** is a process that destroys or removes all forms of microbial life and is carried

out in health-care means by physical or chemical methods.

2. **Disinfection:** is a process that removes many or all pathogenic microorganisms, except

bacterial spores, on nonliving objects.

Methods of Sterilization:

There are two basic methods of sterilization:

1- Physical Methods.

2- Chemical Methods.

1. Physical Methods:

a- Heat:

Heat is the most widely used and reliable method of sterilization, involving destruction of enzymes and other essential cell components. There are two types of heat sterilization:

1. Dry Heat Sterilization:

It is uses higher temperatures in the range of 160-180 °C and requires exposures time up to 2 hours, depending upon the temperature used. It is appropriate for sterilizing glassware and metal surgical instruments. Examples of dry heat sterilization are:

- Incineration (burning). - Red heat. - Flaming.
- Hot air oven: in this method; objects to be sterilized are exposed to high temperature (160°C) for period of one hour in an oven. **Advantages:** it is an effective method of sterilization of heat stable objects. The objects remain dry after sterilization. This is the only method of sterilizing oils and powders. **Disadvantages:** takes longer time.

2. **Moist Heat Sterilization:** it is involves the use of steam in the range of 121-134 °C. Steam under pressure is used to generate high temperature needed for sterilization. Moist heat may beused in three forms to achieve microbial inactivation:

- Pasteurization (at temperature below 100 °C):

This process was originally used by Louis Pasteur. This procedure is used in food and dairy industry. There are two methods of pasteurization, the holder method (heated at 63 °C for 30 minutes) and flash method (heated at 72 °C for 15 seconds) followed by quickly cooling to 13°C.

- Boiling (at temperature 100 °C):

Boiling water (100 °C) kills most vegetative bacteria and viruses immediately.

- Autoclaving (at temperature above 100 °C):

It is use pressurized steam to destroy microorganisms and is the most dependable system available for the decontamination of laboratory waste and the sterilization of laboratory glassware, media and reagents. The conditions of sterilization in an autoclave are 15 minutes, 15 psi at 121°C.

b- Filtration:

It is a process does not destroys but removes the microorganisms. It is used for both the clarification and sterilization of liquids and gases as it is capable of preventing the passage of both viable and non-viable particles. Sterilizing grade filters are used in the treatment of heat sensitive injections and ophthalmic solutions, biological products and air.

c- Radiation:

Two types of radiation are used, ionizing and non-ionizing. Non-ionizing rays are low energy rays with poor penetrative power while ionizing rays are high energy rays with good penetrative power. Since radiation does not generate heat, it is termed “cold sterilization”.

- **Non-ionizing rays:** are rays of wavelength longer than the visible light. Microbicidal wavelength of UV rays are in the range of 200-280 nm, with 260 nm being most effective.
- **Ionizing rays:** are of two types, particulate and electromagnetic rays. Electron beams are particulate in nature while gamma rays are electromagnetic in nature.

2 .Chemical Methods:

Sterilants are chemicals that destroy pathogenic bacteria from nonliving surfaces. Some chemicals have a very narrow spectrum of activity and some have a very wide one. Those chemicals that can sterilize are called chemosterilants. Those chemicals that can be safely applied over skin and mucus membranes are called antiseptics.

Classification of Disinfectants:

1- Based on consistency:

- Liquid (e.g., alcohols, phenols).
- Gaseous (e.g. formaldehyde vapor, ethylene oxide)

2- Based on mechanism of action:

- Action on membrane (e.g., alcohol, detergent).
- Denaturation of cellular proteins (e.g., phenol).
- Oxidation of enzymes (e.g., H_2O_2, halogens).
- Damage to nucleic acids (e.g., formaldehyde).

Some Disinfectants with Their Applications:

1. Alcohols:

Mode of action: alcohols dehydrate cells, disrupt membranes and cause coagulation ofprotein.
Examples: ethyl alcohol, isopropyl alcohol.
Application: 70% aqueous solution is more effective at killing microbes than absolutealcohols. 70% ethyl alcohol (spirit) is used as antiseptic on skin.
Disadvantages: skin irritant, volatile (evaporates rapidly), inflammable.

2. Aldehydes:

Mode of action: acts through alkylation of amino-, carboxyl- or hydroxyl group and probably damages nucleic acids.

Examples: formaldehyde.

Application: 40% formaldehyde (formalin) is used for surface disinfection and evaporation ofrooms. Evaporation is achieved by boiling formalin.

Disadvantages: vapors are irritating (must be neutralized by ammonia), has poor penetration, leaves non-volatile residue.

3. Phenols:

Mode of action: act by disruption of membranes, precipitation of proteins and inactivation of enzymes.

Examples: 5% phenol, chloroxylenol (Dettol).

Application: Joseph Lister used it to prevent infection of surgical wounds. They act as disinfectants at high concentration and as antiseptics at low concentrations. They are bactericidal, fungicidal, mycobactericidal but are inactive against spores and most viruses.

Disadvantages: it is toxic, corrosive and skin irritant.

4. Surface Active Agents:

Mode of actions: they are disrupt membrane resulting in leakage of cell components.

Examples: these are soaps or detergents. Detergents can be anionic or cationic. The anionicdetergents include soaps. Cetrimide act as cationic detergents.

Application: they are active against vegetative cells, mycobacteria and enveloped viruses.They are widely used as disinfectants at dilution of 1-2%.

Laboratory Safety:

The Occupational Exposure to Hazardous Chemicals in Laboratories Standard:

The provisions in the occupational Exposure to hazardous chemicals in laboratories standard cover the routes of exposure, chemical inventory, storage of chemicals and chemical spills.

1. Routes of Exposure:

There are several ways that a hazardous chemical can enter the body. Hazardous chemicals can enter through the mouth or a cut on the hand, but also through the lungs or eyes. Some hazardous chemicals can enter the body through intact skin.

2. Chemical Inventory:

Laboratories keep an inventory of all chemicals used for testing and other procedures. A chemical inventory is useful in other ways. For example, laboratories share their chemical inventory with the local fire department, so that fire department personnel can come prepared to resolve a hazardous chemical spill, an explosion, or another type of chemical emergency.

3. Storage of Chemicals:

Storage of chemicals is important to ensure not only the safety of the individuals working in a laboratory but also the safety of others in the same building.

Rules for Store Chemicals:

• Store similar chemicals together to minimize interactions between chemicals.

• Keep flammable chemicals together in a flammable-storage cabinet.

• Store hazardous chemicals separately from nonhazardous chemicals.

• Check for improperly stored chemicals, leaking containers.

4. Chemical Spills:

Although most individuals are extremely careful when handling chemicals, chemical spills are inevitable. The person spilling the chemical should take responsibility for cleaning it up. Notify the supervisor and report the spilled chemical and location.

The Bloodborne Pathogens Standard:

The bloodborne pathogens standard was issued in 1991 and most recently updated in 2012. Its purpose is to protect workers from microbiological pathogens that are carried in blood and body fluids.

Requirements of the Bloodborne Pathogens Standard:

1. Train workers before they are exposed to blood and body fluids.

2. Offer each employee the hepatitis B vaccination series.

3. Provide appropriate PPE (personal protective equipment) such as gloves, laboratory coats,face shields, and goggles, and instruct personnel on when and how to use this equipment.

Clinical Laboratories and Biosafety Levels:

Clinical laboratories represent a unique working environment because not only hazardous chemicals but also biological hazards are part of the environment. Biosafety levels are used to describe the potential biological hazard and the function of the laboratory. There are four biosafety levels, with 1 being the safest environment and 4 being the most hazardous environment.

1. Biosafety Level 1:

A biosafety level 1 laboratory contains equipment, practices, and facilities that will be used with organisms that do not consistently cause disease in healthy adults.

2. Biosafety Level 2:

A biosafety level 2 laboratory contains equipment, practices, and facilities used to identify and characterize moderate- risk agents derived from the community that cause disease in immunocompromised and immunocompetent people.

3. Biosafety Level 3:

A biosafety level 3 laboratory contains equipment, practices, and facilities used to identify and characterize organisms that cause severe or potentially lethal infections transmitted through inhalation.

4. Biosafety Level 4:

A biosafety level 4 laboratory contains equipment, practices, and facilities used to identify and characterize extremely hazardous infectious organisms, usually transmitted through aerosols, that frequently cause fatal diseases, diseases with no cure or treatment, or diseases with an unknown transmission mechanism.

Laboratory Safety Equipment:

1. Biological safety cabinet.

2. Fume hood.

3. Needle stick prevention engineering control.

4. Fire suppression systems.

5. Pipetting aids.

6. Eye wash stations.

7. Emergency showers.

الأسبوع الرابع

الهدف التعليمي: التعرف على العينات وأنواعها وطرق جمعها وكيفية التعامل معها.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Sample Collection:**

Samples Types:

A wide variety of samples types may be collected:

- Blood and blood fractions (plasma, serum, buffy coat, red blood cells).
- Urine.
- Feces.
- Semen.
- Saliva/buccal cells.
- Cerebrospinal fluid (CSF).
- Tissue (from surgery, autopsy, transplant).
- Placental tissue, meconium, cord blood.
- Bone marrow.
- Breast milk.
- Bronchoalveolar lavage.
- Cell lines.
- Exhaled air.
- Fluids from cytology (ascites, pleural fluid, synovial fluid, etc.).
- Hair.
- Nail clippings.

Collection procedures:

Collection procedures will vary according to sample type and the required analyses.

1- Blood Collection:

Blood can be collected from three different sources:
a- Venous blood. b- Arterial blood. c- Capillary blood.

a- Venous Blood: it is the most commonly required blood sample. The majority of routine tests are performed on it. This is obtained directly from the vein. Best site for the collection of venous blood is the deep veins of the antecubital fossa.

Select Venipuncture Site:

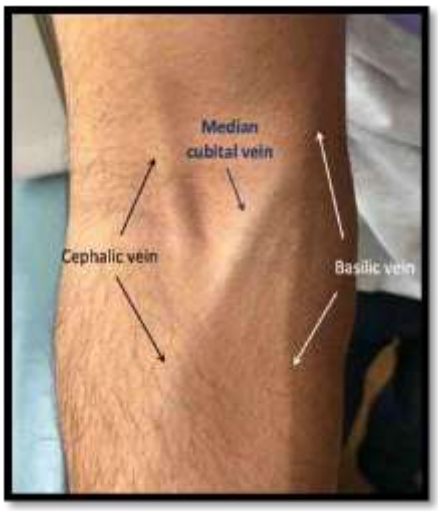
In most cases, blood will be drawn from an arm vein, since these vessels are usually large, close to the skin surface, and easy to penetrate. An alternate sites must be selected because of burns, amputation, or other medical consideration. Alternate sites include the back of the hand,ankle, or foot.

Materials:

- Tourniquet.
- Disposable sterile needle.
- Cotton swap.
- Alcohol.

Procedure:

- If multiple samples are to be collected, it is better to use a butterfly needle.
- Palpate the vein.
- After all the precautions have been made and the patient is comfortable, a tourniquet should be applied on the upper arm.
- The area to be punctured should be sterilized with a spirit swab and allowed to dry for awhile.
- Insert the needle into the vein in such a way that it traverses a little distance under the skin .
- Do not enter the vein directly and vertically, as chances of puncturing the other side of thevenous wall.
- Now draw the blood according to your requirement.
- After this, first remove the tourniquet and place a clean spirit swab at the site of vein-puncture and with draw the needle.
- Put the blood into a suitable tube, already labeled.



b- Arterial blood: it is used for the estimation of blood gases. This sample is occasionally required.

c- Capillary blood: it is a great value in children and adult with difficult veins. Select a suitable site for puncture, the ball of the finger or the side of the thumb. Form a baby, it is bestto obtained blood from the base of the heel, only few tests can be performed.

Materials:

- Cotton swab. - Alcohol. - Disposable lancet. - Capillary tube.

Procedure:

- Rub the area by massaging.
- Sterile the area with 70% alcohol by the cotton swab and allow to dry.
- Punch the area by disposable lancet, the puncture should be about 3 mm.
- Wipe off the first drop of blood and a little pressure is applied.
- Never press on blood.
- Take the blood by capillary tube .
- Apply slight pressure over the area.
- Sterile the area with 70% alcohol by the cotton swab.

2- Urine collection:

Many analytes, such as steroids, hormones, and a wide variety of drugs and their metabolites, can be measured in urine, making it a appropriate sample for a variety of tests. Urine collection can performed under several conditions, depending on the required test:

a- First morning samples: they are collected in the cup immediately upon rising in the morning, recommended for analytes requiring concentration for detection in laboratory assays.

b- Random urine samples: they are appropriate for drug monitoring and cytology tests.

c- Fractional samples: the patient fasts after the last evening meal, and the urine is collected in the cup. These samples are used to compare urine analyte levels with their concentrations inblood.

Labeling of Samples:

It is very important to label the following on the sample and request from patient:
- Name of patient.
- Ward which patient in it (number).
- The bed that patient sleep on it (number).
- Date.
-Age.

Sample Transport:

It is refers to the process of moving biological or chemical samples from their collection point to a laboratory for analysis, ensuring the sample's integrity throughout the process. This involves careful handling , packaging, and adherence to regulations to prevent contamination or degradation, ultimately guaranteeing accurate test results

Key Aspects of Sample Transport:

1- Maintaining Sample Integrity: the primary goal is to preserve the sample's original state, preventing any changes that could affect test outcomes.

2- Proper Packaging: this often involves triple packaging , including a leak-proof primary container, a durable secondary container, and a sturdy outer packaging.

3- Temperature Control: many samples , like blood or serum , require specific temperature conditions (e.g., refrigeration or freezing) during transport.

4- Adherence to Regulations: transport often needs to comply with regulations from various bodies, including national transport agencies, IATA, and others, to address potential hazards.

5- Minimizing Hazards: procedures are in place to minimize the risk of infection or contamination during transport, particularly for potentially infectious samples.

6- Specialized Systems: some laboratories use automated systems like pneumatic tube systems or rail-based transport for efficient and safe movement of samples.

Sample Preparation:

It is a crucial step in any analytical method. It involves transforming a sample into a state that is suitable for analysis , ensuring the sample accurately represents the material being studied , and minimizing potential interferences. This process can include a variety of techniques like extraction, filtration, dilution, and chemical treatment, depending on the specific analysis and sample type.

Key aspects of sample preparation:

1- Extraction: isolating the analyte of interest from the sample matrix.

2- Purification: removing interfering substances that can hinder analysis.

3- Concentration: adjusting the analyte concentration to be within the detection range of the analytical instrument.

4- Homogenization: ensuring the sample is uniform, especially for solid samples.

5- Interference removal: eliminating substances that can interfere with the analysis.

الأسبوع الخامس

الهدف التعليمي: التعرف على أنواع الأوساط الزرعية وتحضيرها.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Culture Media:**

Culture media are specific mixtures of nutrients and other substances that support the growth of microorganisms such as bacteria and fungi (yeasts and molds).

Uses: isolate, identify and study the characteristics of microorganism.

Types of Culture Media:

Bacterial culture media can be classified based on consistency, nutritional component and its functional use or application.

1. Based on Consistency:

Liquid Media:

In liquid media (without agar), bacteria grow producing turbidity, surface pellicle , granular deposits. Culturing bacteria in liquid media has some problems: properties of bacteria are not visible in liquid media and presence of more than one type of bacteria cannot be detected.

Solid Media:

Any liquid medium can be solid by the addition of certain solidifying agents such as agar (1.5-2%).

Agar: is the most commonly used solidifying agent. It is an unbranched polysaccharide obtained from the cell membranes of some species of red algae such as the genera Gelidium. It is composed of two long-chain polysaccharides (70% agarose and 30% agaropectin). It is melts at 95 ^{o}C and solidifies at 42 ^{o}C, doesn't contribute any nutritive property. It is not hydrolyzed by most bacteria and it is usually free from growth promoting or growth slow down substances.

Semi-solid Media:

Reducing the amount of agar to 0.2-0.5% to obtain a semi-solid media. Such media are soft and are useful in bacterial motility test. Certain transport media such as Stuart's and Amies media are semi-solid in consistency.

Biphasic Media:

Sometimes, a culture system consist of both liquid and solid media in the same bottle. This is known as biphasic media. The inoculum is added to the liquid medium and when subcultures are to be made, the bottle is simply slanted to allow the liquid to flow over the solid medium.

2. Based on Nutritional Component:

Simple Media: such as peptone water and nutrient agar which can support most non-fastidious bacteria.

Complex Media: such as blood agar have ingredients whose accurate components are difficult to estimate.

Synthetic or Defined Media: such as Davis and Mingioli media are specially prepared mediafor research purposes where the composition of every component is well known.

Note: the bacteria that are able to grow with minimal requirements are said to **non-fastidious** and those that require extra nutrients are said to be **fastidious**.

3. Based on Functional Use or Application:

Basal Media: are basically simple media that supports most non-fastidious bacteria. Peptone water, nutrient broth and nutrient agar considered basal media.

Enriched Media: are used to grow nutritionally fastidious bacteria. Addition of extra nutrients in the form of blood, serum, egg yolk etc., to basal medium makes them enriched media. Blood agar, chocolate agar, Loeffler's serum slope etc. are few of the enriched media.

Blood Agar: is prepared by adding 5-10% blood (by volume) to a basal medium such as nutrient agar or other blood agar bases. After the blood agar base is autoclaved, blood is addedto the medium at temperature just above the solidifying point of agar. The mixture is then poured in the plates and allowed to solidify. Blood agar is useful in detection hemolytic properties of certain bacteria.

Selective Media: are designed to inhibit unwanted commensal or contaminating bacteria and help to select pathogen from a mixture of bacteria. Various approaches to make a medium selective include addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these. MacConkey's agar used for enterobacteriaceae members contains bile salt that inhibits most gram-positive bacteria.

Differential Media: distinguish one microorganism type from another growing on the same medium. This type of media uses the biochemical characteristics of a microorganism growing in the presence of specific nutrients or indicators (such as neutral red, phenol red or methyleneblue) added to the medium to visibly indicate the defining characteristics of a microorganism e.g.: MacConkey's agar, CLED agar, TCBS agar, XLD agar etc.

When a particular substrate (carbohydrate) is inserted into a medium and a mixture of bacteria inoculated on it, only that bacterium that can ferment it produces acid. This change in pH is detected by using a pH indicator inserted in the medium and the bacterium that can ferment the sugar appears in a different color. This approach is used in MacConkey's agar, CLED agar, TCBS agar, XLD agar etc.

MacConkey's agar is the most commonly medium used to culture and identify gram negative bacilli (especially enterobacteriaceae members). It contains bile salts (selective agent), lactose (sugar), peptone and neutral red (pH indicator), agar and water. Those bacteria that can ferment lactose produce pink colored colonies where non-lactose fermenting colonies produce colorless colonies.

Transport Media: such media prevent drying of sample, keep the viability of all organisms inthe sample without altering their concentration. Some of these media (Stuart's and Amie's) aresemi-solid in consistency.

Anaerobic Media: anaerobic bacteria need reduced oxidation-reduction potential and extra nutrients. Such media may be reduced by physical or chemical means. Boiling the medium used to eject any dissolved oxygen. Addition of 1% glucose, 0.1% thioglycolate, 0.1% ascorbic acid, 0.05% cysteine or red-hot iron filings to obtain a anaerobic medium. Robertson cooked meat that is commonly used to grow Clostridium spp. medium.

Preparation of Culture Media:

Preparing culture media involves a series of steps to create a nutrient-rich environment that supports microbial growth. This process typically includes weighing ingredients, dissolving them in water , adjusting pH , sterilizing the media , and dispensing it into appropriate containers like Petri dishes or flasks.

1- Ingredient Selection and Measurement:

a- Choose the appropriate media type:

Consider whether you need a general-purpose media , a selective media (containing ingredients that inhibit the growth of certain microorganisms) , or a differential media (allowing differentiation between different types of microorganisms).

b- Weigh the necessary ingredients:

Use a balance to accurately measure the required amounts of each component , such as (peptones, yeast extract, salts, and agar) for solid media.

c- Dissolve the ingredients:

Add the weighed ingredients to a flask or beaker containing distilled water or a buffer solution.

d- Mix thoroughly:

Stir the solution until all ingredients are completely dissolved . Some media may require gentle heating to aid dissolution.

2- pH Adjustment:

a- Check the pH: use a pH meter to measure the acidity or alkalinity of the media.

b- Adjust the pH: add appropriate acids or bases (e.g., HCl , NaOH) to bring the pH to the desired level for microbial growth. The optimal pH is typically around 7 for bacteria and slightly lower for fungi.

3- Sterilization:

a- Autoclaving: this is the most common method for sterilizing culture media. The media is heated under pressure at 121°C for 15-20 minutes.

b- Filtration: for heat-sensitive media, filtration using a membrane filter with a pore size of 0.22 μm or 0.45 μm is used to remove microorganisms.

4- Dispensing and Storage:

a- Pour into Petri dishes: if preparing solid media , carefully pour the sterilized media into sterile Petri dishes, ensuring an even layer.

b- Dispense into tubes or flasks: for liquid media, dispense the sterilized media into sterile test tubes or flasks for storage or further use.

c- Label and store: label the Petri dishes or tubes with the media type , date , and any other relevant information . Store the media in a cool , dark place or at a specified temperature (e.g., 4°C).

5- Quality Control:

a- Visual inspection: before use, visually inspect the media for any signs of contamination (e.g., cloudiness, particulate matter).

b- Sterility testing: in some cases , sterility testing may be performed to confirm that the media is free of microorganisms.

الأسبوع السادس

الهدف التعليمي: التعرف على عينة البول والفحص المجهري والفيزيائي والكيميائي لعينة البول.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Urine Sample:**

Urine: is a liquid that formed in the kidneys, it is a product of ultrafiltration of plasma by therenal glomeruli.

General Urine Examination (GUE):

1- Macroscopic Examination (Physical Examination and Chemical Examination).2- Microscopic Examination.

Physical Examination:

a- Color:

Normal----------pale to deep amber.

Milky----------chyluria, UTI (urinary tract inflammation).

Reddish----------bleeding in UTI.

Greenish-yellow, dark-brown, orange-yellow----------jaundice.

b- Odor:

Normal---------- aromatic.

Abnormal---------- non aromatic.

c- Specific gravity: density of a substance compared to the density of water.Normal---------- 1.005 to 1.030.

Increased ---------- dehydration, diabetes.

Decreased ---------- kidney disease, diuretic, increased fluid intake.

Chemical Examination:

a- pH: is a measure of acidity or alkalinity of

urine.Normal---------- 5.5 to 8.0.

Affected by:

- Diet.
- Medications.
- Kidney diseases.
- Starvation.

b- Protein:

Normal---------- absence.

Abnormal-------- presence (proteinuria).

b- Glucose:

Normal---------- absence.

Abnormal-------- presence (diabetes).

c- Ketones: end product of fat

metabolism.Normal absence.

Abnormal--------presence (diabetes, fasting, dieting, high fat diet).

d- Blood:

Normal----------absence.

Abnormal------ presence (injury, infection, menstruation, kidney diseases).

e- Bilirubin: break down product of

hemoglobin.Normal absence.

Abnormal------ presence (liver diseases).

Microscopic Examination:

Procedure:

a- Centrifugation:

- Shake the urine sample to make the sample homogeneous.
- Put 10 ml of urine sample into test tube.
- Centrifuge the urine sample for 5 minutes at 2000 RPM and discard from the liquid part.

b- Examination:

- Place a drop of sediment on a glass slide and cover the drop with cover slide.
- Place the glass slide on the microscope stage.
- Examine for the elements: WBCs, RBCs, epithelial cells, yeast, bacteria, mucous, crystals, pus and casts.

Urine Culture:

A urine culture is a laboratory test used to detect and identify bacteria or other microorganisms in a urine sample, which can help diagnose and manage urinary tract infections (UTIs) and other conditions. It involves growing microorganisms from a urine sample to determine if they are present and to identify the specific type and quantity.

- A urine sample is collected , usually via the "clean-catch" method (midstream urine) to minimize contamination.
- The urine sample is placed in a substance that promotes the growth of microorganisms:

a- If no microorganisms grow, the culture is negative.

b- If microorganisms grow , they are identified and tested to determine antibiotic susceptibility.

Urine Sensitivity:

Urine sensitivity , in the context of a urine culture , refers to a laboratory test that determines which antibiotics are most effective at inhibiting or killing bacteria found in a urine sample . This helps doctors choose the right medication to treat a urinary tract infection (UTI).

A urine culture is a lab test that grows and identifies bacteria present in a urine sample. After bacteria are identified , they are tested against different antibiotics to see which ones. The sensitivity test helps doctors select the most appropriate effectively stop their growth antibiotic to treat a UTI , ensuring the infection is effectively treated and reducing the risk of antibiotic resistance.

الأسبوع السابع

الهدف التعليمي: التعرف على عينة البراز والفحص العام لها.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Stool Sample**

General Stool Examination:

General Stool Examination (GSE) is carried out in laboratories for various diagnostic purposes. Examination of stool is very helpful in the diagnosis of disease of the gastrointestinal tract. Mostly a clean container which does not contain any detergent or disinfectant is sufficient for all types of stool examinations including stool culture. It consist the following tests:-

a. Physical Examination of Stool:

Sample should be examined immediately after collection. Samples left standing prolonged will deteriorate helminthes, Ovum, other parasites and increase the numbers of monilia and bacteria which gives wrong results, however the following aspects of stool should be examined:

1- Quantity: the adult person excretions about 150-250 gm. /day of feces, about (1/3- 1/2) of feces dry weight is bacteria.

2- Consistency and form: normal stool is well formed. But in constipation (Dehydration) the stool is solid (Hard) and the semi-solid (soft or loose) seen when taking certain medications and laxatives. In abnormal cases such as diarrhea and dysentery the stool appear liquid, or watery in nature. In cholera the stools have a rice water appearance. In cases of malabsorption of fats the stools are pale bulky and semi-solid.

3- Color:

- Normal colors of stools are light to dark brown due to the Presence of bile pigments.
- Dark black: in cases with bleeding into the intestinal tract the stools become dark tarry in nature due to the formation of acid hematin , if the bleeding is in the small intestines. In case of bleeding in large intestines or rectum stool color may be bright red due to fresh blood.

- Red color: resulted from eating certain colorful foods such as red beets.
- Clay color: the stool may be clay colored due to absence of stercobilinogen in biliary tract obstruction.

4- Odor: the normal fecal odor of stool resulted from indole and skatol. Odor of stools may become offensive in conditions like, Intestinal amoebiasis. In cases of bacillary dysentery and cholera the stools are not foul smelling due to the absence of fecal matter.

5- Blood:

1- The blood is present on the outer surface of the feces and this caused either by contamination from menstrual cycle blood in women or bleeding hemorrhoids from the blood vessels.

2- Blood should be noted in stools if present as it is indicative of Ulceration or presence of any other pathology like malignancy.

6- Mucus: Is present in certain conditions like amoebic or bacillary dysentery.

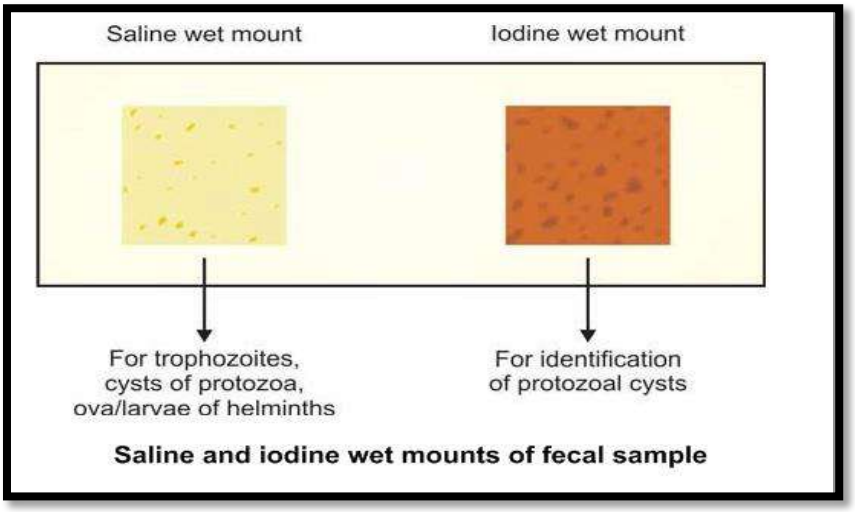
7- Parasite: Stools may contain adult helminthes. Nematodes like Ascaris are easily visible as their size is large. Hook worms and Proglottids of cetodes may also present. These may be visible to the naked eye.

b. Microscopic Examination of Stool:

The laboratory diagnosis of most parasitic infections is by the demonstration of ova of the parasite in the stools of the infected person. The stool is collected in a clean container. The stool can be examined by the following techniques: wet mounts examination and iodine examination.

Saline wet mount examination: the stool is emulsified in normal saline and a large drop is placed on a glass slide and is then covered with a cover slide. Then examined under a light microscope, it is important to examine specimen under 10X objective lens at first to observe large molecules, cells, ova and helminthes, then to the 40X objective to complete the test. It is preferable to keep the condenser down and the intensity of the light low for proper visualization of the ova and cysts. The thickness of the film should be such that one is able to see the printed letters of the newspaper through it.

Iodine examination: Iodine preparation leads to better visualization of morphological details of ova and cysts as it stains the glycogen in them. However it has the disadvantage that the live trophozoites of *Entamoeba histolytica* and other live parasites cannot be seen as the iodine kills them. The examination instructions in normal saline must be followed the same in iodine test.



Saline and iodine wet mounts of fecal sample

Microscopic examination include the following:

(1) Pus cells: Observed in stool the same procedure as in urine.

(2) RBCs: Observed in stool the same procedure as in urine.

(3) Monilia: Observed in stool the same procedure as in urine.

(4) Protozoa: (a) *Entamoeba histolytica*: to investigate the vegetative phase (trophozoite) and cyst, causing amoebic dysentery disease. **(b)** *Entamoeba coli*: trophozoite + cyst *Note:* - most of children diarrhea less than 2 years cause by *Entamoeba coli*. **(c)** *Giardia lamblia*, trophozoite + cyst, Cause watery diarrhea disease in children, especially. **(d)** *Balantidium coli*, trophozoite + cyst, causing Balantidiasis in colon.

(5) Worms : (a) *Enterobius vermicularis* (pinworm): investigating the eggs that are of convex and flat surface and a pointed end. **(b)** *Ascaris lumbricoides:* investigating for eggs which characterized by the content of granular yellow to Brown irregular albumin membrane. **(c)** Hookworm (*Ancylostoma duodenale)*: investigating the eggs where the egg yolk is divided and surrounded by a thin membrane. **(d)** Tapeworms, (Taenia *solium)*: investigating the worm pieces called (gravid segments or Proglottids) that comes out with the feces. **(e)** *Schistosoma mansoni*: Investigating the eggs distinct by lateral spin.

c. Chemical Examination of Stool:

1- pH: the pH of stools is acidic in amoebic dysentery and is alkaline in bacillarydysentery.

2- Occult blood: presence of blood in feces which is not apparent on gross inspection and which can be detected only by chemical tests is called as occult blood. Causes of occult blood present in a number of diseases including malignancy of the gastrointestinal tract.

The reagents used are:

1- Benzidine reagent: development of blue colour is indicative of presence of occult blood in the stool specimen.

2- Orthotolidine: development of green colour Benzidine test is also highly sensitive and false-positive reactions are common. Since bleeding from the lesion may be intermittent, repeated testing may be required.

d. Stool Culture:

Procedure:

Stool is cultured by taking a sample by loop and cultured on different types of culture media according to the type of bacteria or diagnosis of case investigated as follows:

1- It is cultured on thiosulfate citrate bile salts sucrose agar media if the patient is suspected of cholera infection.

2- It is cultured on Lowenstein-Jensen medium for *Mycobacterium tuberculosis* if the person suspected of gastrointestinal tuberculosis.

3- It is cultured on blood agar if suspected of infection with *Staphylococcus aureus* which it is blood hemolytic.

4- It is cultured on MacConkey agar medium to detect lactose fermentation bacteria in pink color colonies include (*E .coli*, *Klebsiella* and *Enterobacter*), but if it's not lactose fermenter, it is either *Proteus* which is identified by (diffusion phenomenon), or *pseudomonas* which is identified by (pyocynin test) and to distinguish between *Shigella* and *Salmonella*, by using serological and biochemical test.

Stool Sensitivity:

A stool sensitivity test , also known as a stool culture and sensitivity test , is a laboratory procedure used to identify harmful bacteria or other microorganisms in a stool sample and determine which antibiotics are effective against them. This test is often performed when a person experiences gastrointestinal symptoms like diarrhea , abdominal pain , fever , or vomiting , to determine the cause and guide appropriate treatment . The identified micro-organisms are exposed to various antibiotics to see which ones effectively inhibit their growth.

الأسبوع الثامن

الهدف التعليمي: التعرف على السائل المنوي وطرق فحصه.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Seminal Fluid**

Semen (or seminal fluid) is a fluid that is emitted from the male genital tract and contains sperms, sugar and protein substances that are capable of fertilizing female ova. Semen analysis, include two major tests:

a- Macroscopic or Physical examination:

Physical Examination is carried out after liquefaction of semen that occurs usually within 20-30 minutes of ejaculation.

1) Volume:

Volume of ejaculated semen sample should normally be more than 2 ml. It is measured after the sample has liquefied. Volume less than 2.0 ml is abnormal, and is associated with low sperm count. Excess Volume more than 5 ml. could also mean diluted amount of sperm present in ejaculum.

2) Color:

Normal semen is viscous and opaque-white (milky) or opaque gray-white in color. After prolonged abstinence , it appears slightly yellow. But In case of an inflammatory purulent appears yellow. Sometimes appear brown in color in cases of bleeding from a blood capillaries.

3) Viscosity:

Normal semen is thick and viscous immediately following ejaculation, it becomes liquefied within (10 to 30) minutes at (37 °C) by the action of proteolytic enzymes secreted by prostate and turned into a watery consistency, helps sperm to move. It is considered abnormal if liquefaction does not occur within 60 minutes.

The viscosity of the sample is assessed by filling a pipette with semen and allowing it to flow back into the container. Normal semen will fall drop by drop. If droplets form 'threads' more than 2 cm long, then viscosity is increased.

Increased semen viscosity affects sperm motility and leads to poor invasion of cervical mucus; (it results from infection of seminal vesicles or prostate).

Report is written as follows:

Liquefaction within 30 min. at 37 °C

or in case of delayed liquefaction is written:

Viscous after 1 hr. incubation at 37 °C

4) pH:

A drop of liquefied semen is spread on pH paper (of pH range 6.4-8.0) and pH is recorded after 30 seconds. Normal pH is (7.2 to 8.0) after 1 hour of ejaculation.

The portion of semen contributed by seminal vesicles is alkaline, while portion from prostate is acidic.

1- Low pH (< 7.0) with absence of sperms (Azoospermia) suggests obstruction of ejaculatory ducts or absence of vas deferens.

2- High pH is usually associated with low semen volume [as most of the volume is supplied by seminal vesicles (no prostatic fluid)].

b- Microscopic Examination:

The most important test in semen analysis for infertility is microscopic examination of the semen, which include:

1) Sperm Count:

The sperm count is done after liquefaction and the total number of spermatozoa is reported in millions/ml or (106/ml), and there are two methods for sperm count**:**

- Direct method:

This method is done by taking a drop of semen on clean slid and covered with a cover slide and immediately examined under 40× objective lens and each five sperm per microscopic field represents one million sperm per ml or cm^3.

- Haemocytometer:

In this method sperm count is done after liquefaction in a counting chamber **(Haemocytometer)** following dilution with diluting fluid and the total number of spermatozoa is reported in millions/ml or (106/ml).

Note: Semen specimen is incubated at 37°C after collection to insure liquefaction.

Diluting fluid:

Sodium bicarbonate formalin (1 ml formalin + 5 gm. sodium bicarbonate)

Procedure:

1. Semen is diluted 1:20 with diluting fluid, (take 1 ml of liquefied semen in a graduated tube and fill with diluting fluid to 20 ml mark. Mix well), or by using WBC diluting pipette just the same way in WBC total count, and a coverslip is placed over the counting chamber.
2. Counting chamber is filled with the well-mixed diluted semen sample using a Pasteur pipette. The chamber is then sit for 10-15 minutes for spermatozoa to settle.
3. The chamber is placed on the microscope stage. Using the 40× objective and iris diaphragm lowered sufficiently to give sufficient contrast, number of spermatozoa is counted in 4 large corner squares. Spermatozoa whose heads are touching left and upper lines of the square should be counted considered as 'belonging' to that square.
4. Sperm count per ml is calculated as follows: *Sperm count = Sperms counted × correction factor × 1000 ÷ Number of squares counted × Volume of 1 square*
5. Normal sperm count is ≥ 20 million/ml (i.e. ≥ 20 × 106/ml).
6. Sperm count < 20 million/ml may be associated with infertility in males.

Few millions less than 10 called Oligospermia. Zero sperm count (absence of any sperm in the semen) called Azoospermia.

Count / ml = N/4 *10 *1000* 20

N :

The total count in 4 squares

10 :

Volume factor

1000 :

Generally the sperm count by ml or cm^3 so multiply by 1000 to convert mm3 to ml or cm^3

20 :

dilute factor

Count /ml = N * 50,000

Some common abnormal results in semen count:

1. **Azoospermia**: complete absence of sperm from the ejaculate, present in about 1% of all men and 10%-15% of infertile men.

2. **Aspermia**: complete absence of seminal fluid emission upon ejaculation.

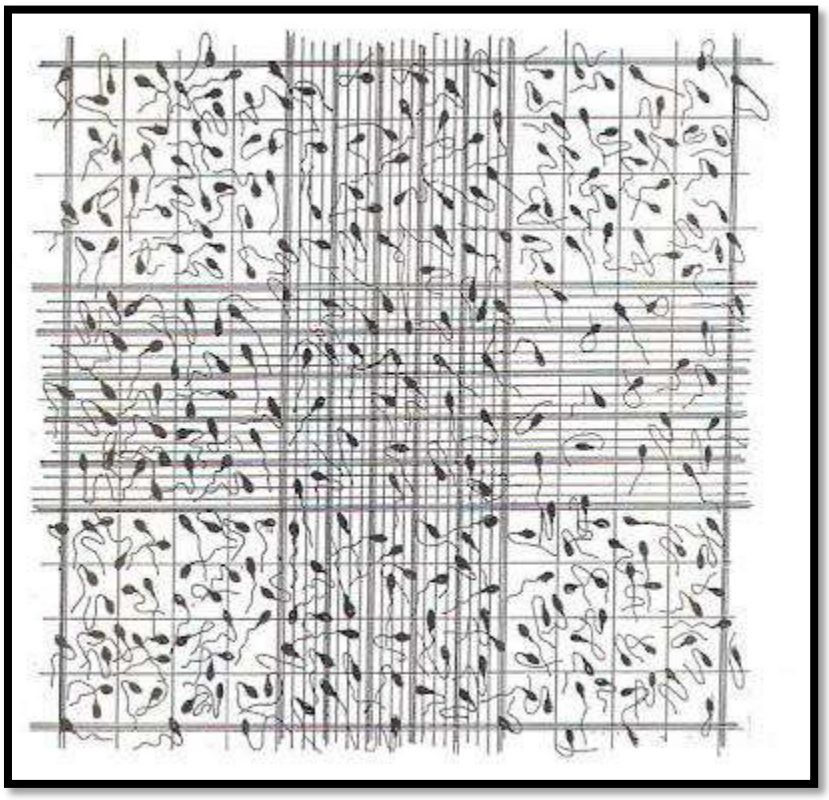
3. **Oligospermia**: low sperm count, defined by the World Health Organization (WHO) as concentrations less than 15 million sperm/ml.

Oligospermia is further classified as:

(a) Mild: concentrations 10-15 million sperm/ml.

(b) Moderate: concentrations of 5-10 million sperm/ml.

(c) Severe: less than 5 million sperm/ml.



Sperm count in haemocytometer

2) Motility or Movement:

The first laboratory assessment of sperm function in a wet preparation is sperm motility (ability of the sperms to move). Sperm motility is essential for penetration of cervical mucus,

traveling through the fallopian tube, and penetrating the ovum. Only those sperms having rapidly progressive motility are capable of penetrating ovum and fertilizing it.

For a normal result, more than 50 percent of sperm must move normally an hour after ejaculation, the sperm motility divided into:

a- Active; rapidly progressive spermatozoa (moving fast forward in a straight line),

b- Sluggish; Slowly progressive spermatozoa (slow linear or non-linear, i.e. crooked or curved movement),

c- Non-progressive spermatozoa (movement of tails, but with no forward progress).

d- Immotile spermatozoa (no movement at all) (WHO criteria).

Sperms of grades (c) and (d) are considered to be poorly motile (Asthenospermia).

Procedure:

A drop of semen is placed on a glass slide, covered with a coverslip that is then ringed with petroleum jelly to prevent dehydration, and examined under 40× objective. At least 200 spermatozoa are counted in several different microscopic fields. Result is expressed as a percentage (%).

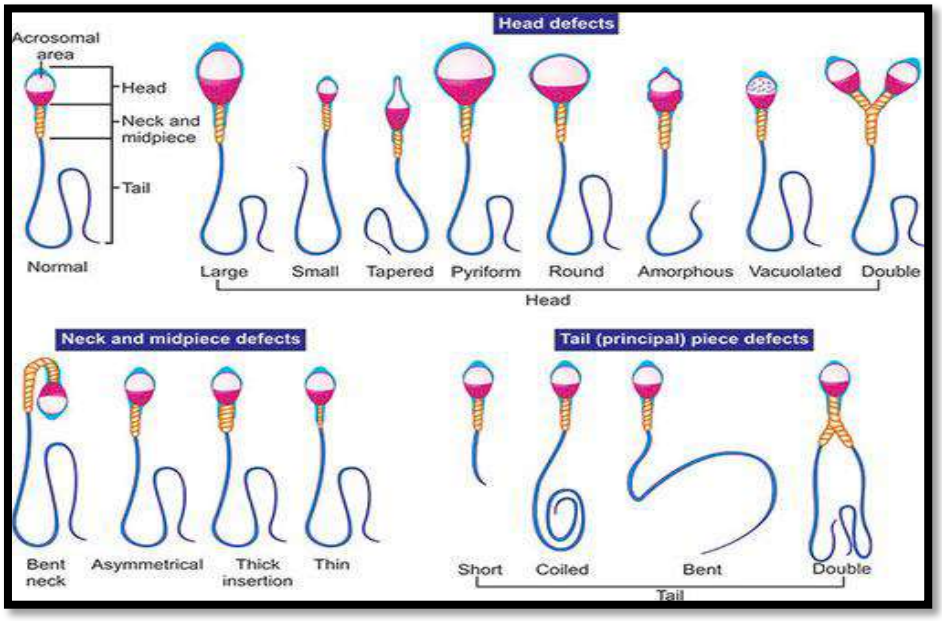
Normal percentage is ≥ 50% of sperms show rapid progressive and slow progressive motility.

3) Sperm Morphology:

A spermatozoa consists of three main components: head, neck, and tail. Tail is further subdivided into midpiece (principle) piece, and end piece. The defects in morphology that are associated with infertility in males include:

- Defective mid-piece (causes reduced motility).
- Incomplete or absent acrosome (causes inability to penetrate the ovum).
- Giant head (defective DNA condensation).

Normal results; more than 50% of spermatozoa should be normal morphology (WHO, 1999).



Procedure:

A smear is prepared by spreading a drop of seminal fluid on a glass slide, stained, and percentages of normal and abnormal forms of spermatozoa are counted. The staining techniques used are hematoxylin-eosin, and rose bengal-toluidine blue stain. At least 200 spermatozoa should be counted under oil immersion. Percentages of normal and abnormal spermatozoa should be recorded.

Teratozoospermia, also known as Teratospermia, is a semen alteration in which there is a large number of spermatozoa with abnormal morphology or (it is a condition characterized by the presence of sperm with abnormal morphology that affects fertility in males).

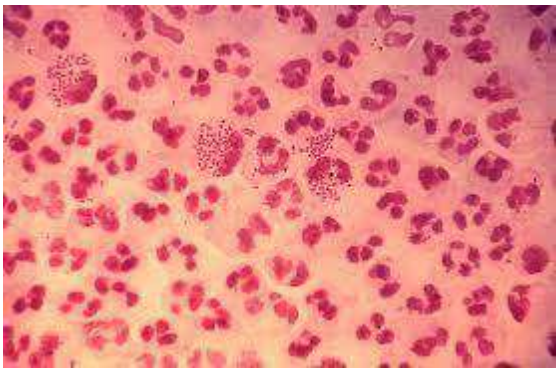
4) Proportion of White Cells:

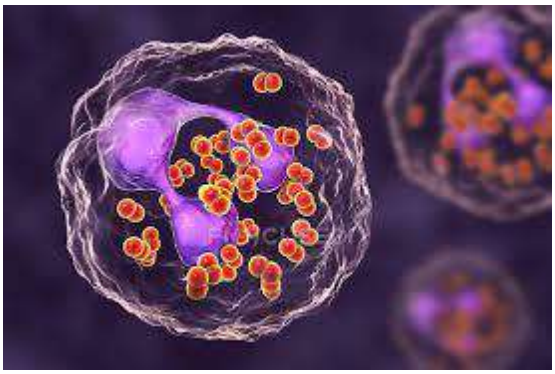
In microscopic examination also investigating the presence of white blood cells (Pus cells) in cases of; Gonorrheal infection, inflammation of the prostate, and sometimes a parasite like *Trichomonas vaginals* also found which is transmitted through sexual intercourse (S.T.D; Sexually transmitted disease).

Round cells on microscopic examination may be white blood cells or immature sperm cells.

It is very important to differentiate between them by using a special stain (peroxidase or papanicolaou) is required to differentiate between them.

- Presence of large number of immature sperm cells indicates spermatogenesis dysfunction at the testicular level.
- Presence of White blood cells more than (1 million/ml) indicate presence of infection, and semen culture and sensitivity is required.





Fructose test:

The semen fructose test is a diagnostic tool used in male fertility assessments to measure the amount of fructose in semen. Fructose, primarily produced by the seminal vesicles, is a crucial energy source for sperm. The test helps evaluate the function of the seminal vesicles and can be indicative of potential issues with male fertility, particularly when azoospermia (absence of sperm) is present.

Procedure:

1- Abstinence: the individual should abstain from sexual activity for 2-7 days before the test.

2- Sample collection: a semen sample is collected in a sterile container , either through masturbation or with a special condom during intercourse.

3- Sample handling: the sample should be submitted to the lab within an hour of collection and kept at room temperature until then.

4- Analysis: in the lab, the semen sample is analyzed to measure the fructose concentration , often using spectrophotometry or chemical assays.

5- Result interpretation: the results, typically available in 1-2 days , can help assess the function of the seminal vesicles and overall male fertility.

الأسبوع التاسع

الهدف التعليمي: التعرف على اختبار تلازن الدم.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Hemagglutination Test**

Hemgglutination test detect antibody or antigen and involve agglutination of bacteria, red cells, or antigen- or antibody coated latex particles. Such tests rely on the bivalent nature of antibodies, which can cross-link particulate antigens.

Steps of Hemagglutination:

-Primary phenomenon (sensitization).

-Lattice formation (aggregation stage).

-Tertiary phenomenon.

1- Primary phenomenon (sensitization):

Involve antigen-antibody combination through single antigenic determination on the particle.

2- Lattice formation (aggregation stage):

Represent the sum of interaction between antibody and multiple antigenic determinants on a particle dependent on environmental conditions as well as the concentration of antigen and antibody.

3- Tertiary phenomenon:

Reaction not visible, detected by effect of reaction on tissues or cells.

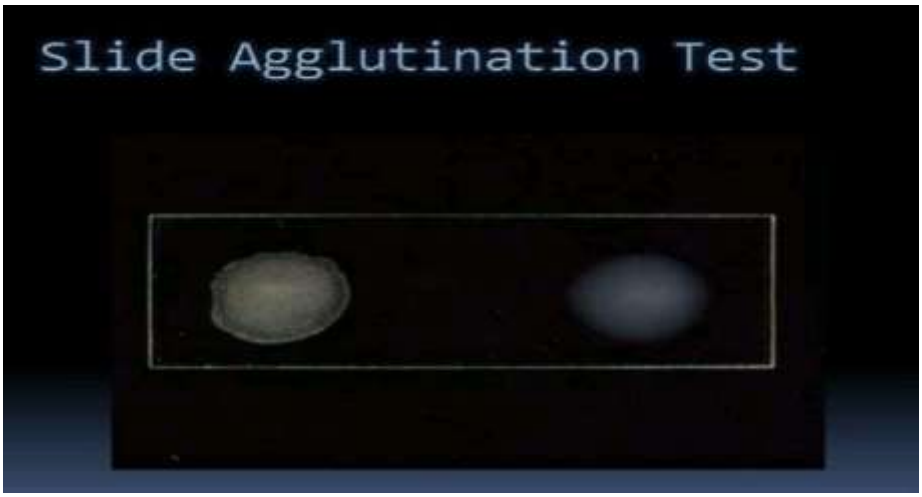
Uses of Hemagglutination:

1- Aid in the identification, by means of known antisera(serum containing antibodies specific for a given antigen),microorganisms cultured from clinical specimens.

2- Help estimate the titer of antibacterial agglutination in the serum of patients with unknown disease.

Methods of Hemagglutination:

Slide agglutination (rapid): add a drop of antiserum , mix with antigen and rock slide for approx. 1minute.



Tube agglutination (slow): standard quantitive method for determination of antibodies . Routinely employed in diagnosis of different types of viruses and bacteria.

الأسبوع العاشر

الهدف التعليمي: التعرف على اختبار الاليزا.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Enzyme-linked immunosorbent assay (ELISA)**

Enzyme-linked immunosorbent assay (ELISA) test is the most widely used type of immunoassay. ELISA is a rapid test used for detecting or quantifying antibody (Ab) against viruses, bacteria and other materials or antigen (Ag). ELISA is so named because the test technique involves the use of an enzyme system and immunosorbent.

ELISA test is being increasingly used in the detection of antigen (infectious agent) or antibody due to its simplicity and sensitivity. It is as sensitive as radioimmunoassay (RIA) and requires only microlitre quantities of test reagents. It has now been widely applied in detection of a variety of antibody and antigens such as hormones, toxins, and viruses.

Materials Needed in ELISA Test:

1. ELISA Readers: need to have appropriate filter (650 nm and 450 nm).

2. Pipette: are available as fixed as well as adjustable volume as well as single channel and multi-channel.

3. Washing system: it can be manual system that washes one row or column at a time or semi-automated systems that wash one strip or plate at a time or fully automated systems that can process multiple plates

4. Reagents: needed for the testing concluded in the kit (coated plates , sample diluents , controls, wash concentrate, conjugate, substrate, stop solution).

ELISA Procedure:

1- Coating the plate: a microtiter plate (usually polystyrene) is coated with a known quantity of either the target antigen (if detecting antibodies) or a capture antibody (if detecting antigens.

2- Blocking: non-specific binding sites on the plate are blocked with a blocking agent (like BSA or casein) to prevent unwanted interactions.

3- Adding the sample: the sample containing the substance to be detected (antigen or antibody) is added to the plate and incubated. If the target substance is present, it will bind to the immobilized molecule.

4- Washing: the plate is washed to remove unbound substances.

5- Adding detection antibody: a detection antibody, often conjugated to an enzyme (like horseradish peroxidase or alkaline phosphatase), is added. This antibody will bind to the target substance (if present).

6- Washing again: the plate is washed again to remove unbound detection antibody.

7- Adding substrate: a substrate for the enzyme is added.The enzyme converts the substrate into a detectable product, often a colored compound.

8- Signal detection: the amount of product formed is measured, usually by a spectrophotometer.The signal intensity is proportional to the amount of the target substance in the sample.

Troubleshooting Steps:

1- Review the protocol: ensure all steps, including reagent preparation, incubation times and temperatures , washing procedures , and substrate usage , are followed precisely according to the protocol.

2- Check reagents: verify that all reagents are within their expiration dates, stored correctly, and prepared according to the instructions.

3- Assess standard curve: a poor standard curve can indicate issues with the standard solution, pipetting errors, or inadequate mixing

4- Evaluate plate washing: inconsistent or insufficient washing can lead to high background or non-specific binding. Ensure thorough washing of plates.

5- Control for background: high background can be caused by non-specific binding , substrate contamination , or incorrect blocking . Optimize blocking buffer, reduce incubation times, and use fresh substrate.

6- Consider incubation conditions: ensure correct incubation temperatures and times are maintained. Temperature fluctuations or prolonged incubation can affect antibody binding.

7- Address pipetting errors: incorrect volumes or inconsistent pipetting can lead to inaccurate results. Ensure proper calibration and technique.

8- Evaluate detection system: check for issues with the detection antibody, conjugate, or substrate. Ensure proper dilutions and incubation times.

9- Optimize assay format: if the assay is not sensitive enough , consider using a more sensitive format like a sandwich ELISA.

10- Address edge effects: edge effects can be caused by temperature variations or evaporation. Ensure proper plate handling and incubation conditions.

11- Consider plate reader settings: verify the correct wavelength settings and plate reader calibration.

12- No signal: check for issues with the standard curve, reagent concentrations, or incubation conditions.

13- Weak signal: increase antibody concentrations, optimize incubation times, or use a more sensitive assay format.

14- High background: optimize blocking buffer , reduce incubation times , and ensure thorough washing.

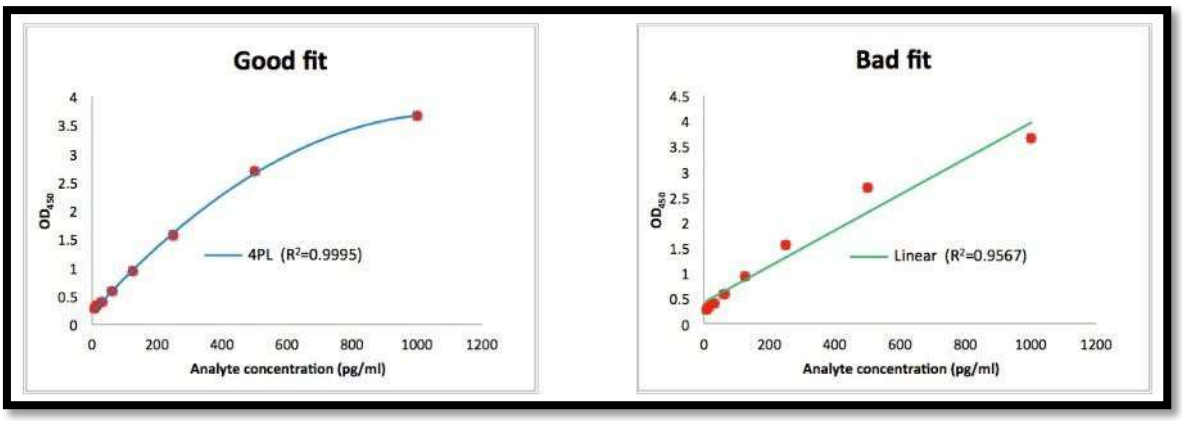
15- Poor duplicates: ensure proper pipetting technique , reagent mixing , and incubation conditions.

Cutoff Value of ELISA:

The cutoff value in an ELISA (Enzyme-Linked Immunosorbent Assay) is a threshold used to classify samples as either positive or negative for a specific analyte. It's essentially a decision point based on the optical density (OD) readings , differentiating between samples that contain the target and those that don't or contain it at very low levels.

ELISA Standard Curve:

An ELISA standard curve is a graph that relates the signal (usually absorbance) from an ELISA assay to known concentrations of a target analyte . It's essential for quantifying unknown sample concentrations by interpolation from the curve. Typically , the curve is sigmoidal (S-shaped) with a linear region that allows for accurate measurement.



الأسبوع الحادي عشر

الهدف التعليمي: التعرف على اختبار المناعة الاشعاعية.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Radioimmunoassay (RIA)**

Radioimmunoassay (RIA), a highly sensitive laboratory technique used to measure minute amounts of substances including antigens, hormones, and drugs present in the body. The substance or antigen (a foreign substance or pathogen in the body that causes antibody production by the B lymphocytes of the body) to be measured is injected into an animal, causing it to produce antibodies. Serum containing the antibodies is withdrawn and treated with a radioactive antigen and later with a nonradioactive antigen. Measurements of the amount of radioactivity are then used to determine the amount of antigen present.

Procedure:

1- Preparation of standards and samples:

- Sample preparation: the sample to be tested is extracted and diluted to ensure it falls within the measurement range of the assay.

- Standard preparation: a known concentration of the antigen (standard) is prepared using a dilution buffer and then serially diluted to create a series of known concentrations for calibration.

2- Antibody fixation:

- Radioactive labeling: a known quantity of the antigen is made radioactive, often by labeling it with a gamma-radioactive isotope like iodine-125 or iodine-13.

- Antibody incubation: a known concentration of the specific antibody is fixed in micro-titration wells.

- Incubation and washing: the radiolabeled antigen is added to the wells and incubated , allowing it to bind to the antibody. Unbound labeled antigen is then washed away, leaving the antibody-bound labeled antigen in the wells.

3- Antigen competition:

- Sample and standard addition: samples of unknown antigen concentration and standard samples are added to separate wells.

- Competition: the unlabeled antigen in the samples competes with the labeled antigen for binding to the antibody. The more unlabeled antigen present, the more labeled antigen will be displaced.

- Washing: unbound labeled antigen is washed away , leaving behind a mixture of bound and unbound labeled antigen.

4- Detection and analysis:

- Radioactivity measurement: the radioactivity of the unbound (free) labeled antigen is measured using a gamma counter.

- Calibration and calculation: the measured radioactivity is compared to a standard curve (generated from the standards) to determine the concentration of the unlabeled antigen in the sample.

Troubleshoot:

1- Sample related issues:

- Concentration: samples may be too dilute or concentrated for the standard curve, requiring dilution or concentration of the sample.
- Matrix effects: the sample matrix (e.g. , serum , plasma , etc.) can affect assay performance. Ensure the standards are prepared in a matrix that closely mimics the patient samples.
- Interferences: certain substances in the sample can interfere with the assay . Consider extraction or purification steps to remove potential interferes, especially if using competitive RIAs.

2- Reagent-related issues:

- Antibody: check antibody quality, low number, and expiration date. Variations in ED50 (the concentration of antibody that binds 50% of the radioligand) can indicate changes in antibody concentration or quality. Ensure the antibody is appropriately diluted for optimal binding of the radioligand.
- Radioligand: low maximum binding and high non-specific binding (NSB) can indicate radioligand damage. Check the radioligand quality or replace it with a fresh, high-purity batch.
- Other reagents: test buffers for contamination, pH changes, or other inconsistencies that can affect the assay.

3- Environmental and equipment factors:

- Temperature: temperature fluctuations can affect reaction rates and binding kinetics. Maintain a consistent temperature throughout the assay.
- Incubation time: inconsistent incubation times can lead to variations in results. Ensure that all wells are incubated for the same duration.
- Equipment: calibrate pipettes and other dispensing equipment regularly. Ensure the gamma counter is properly calibrated and functioning correctly.

- Contamination: cross-contamination can lead to inaccurate results . Use appropriate techniques to minimize the risk of contamination.

4- Assay-specific issues:

- Competitive vs. sequential RIAs: in competitive RIAs , if the antibody has different avidities for labeled and unlabeled antigens, sequential RIAs may be more sensitive.
- Non-specific binding (NSB): high NSB can be minimized by optimizing washing steps and using appropriate blocking agents.
- Interferences in competitive assays: low binding may indicate issues with radioligand , antibody, or assay conditions

الأسبوع الثاني عشر

الهدف التعليمي: التعرف على تقنية التألق المناعي.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Immunofluorescence Technique**

Introduction:

Immunofluorescence is histochemical laboratory staining technique used for demonstrating the presence of Antibodies bound to Antigen in tissues or circulatory body fluids. Immunofluorescence is the labeling of antibodies or antigens with fluorescent dyes.

They are permit early diagnosis, treatment ,and subsequent monitoring of disease activity in patients . Fluorochromes are dyes that absorb ultra-violet rays and emit visible light. This process is called fluorescence. The fluorochromes commonly used in immunofluorescence are fluorescein isothiocyanate (FITC) (green) and tetra methyl rhodamine isothiocyanate (TRITC) (red). Fluorescent techniques involve the emission of light of one color/wavelength and a low energy level from a substance being irradiated with light of a different wavelength

The antibody is linked with fluorescein isothiocyanate (FITC) via a thiocarbamide linkage without destroying its capacity to react with the corresponding antigen.

There are three basic types of immunofluorescence techniques:

1. Direct immunofluorescence **(**DIF).
2. Indirect immunofluorescence (IIF).
3. Microimmunofluorescence (MIF).

Direct immunofluorescence:

This is a one-step histological staining procedure for identifying *in vivo* antibodies that are bound to tissue antigens. This technique is used to detect antigen in clinical specimens using specific fluorochrome labeled antibody.

The steps involved are:

1- Fixation of smear on the slide.

2- Treating with labeled antibody.

3- Incubation, washing to remove unbound excess labeled antibody visualization under fluorescent microscope. When viewed under fluorescent microscope, the field is dark and areas with bound antibody fluoresce green.

4- Ag is fixed on the slide.

5- Fluorescein labeled Ab's are layered over it.

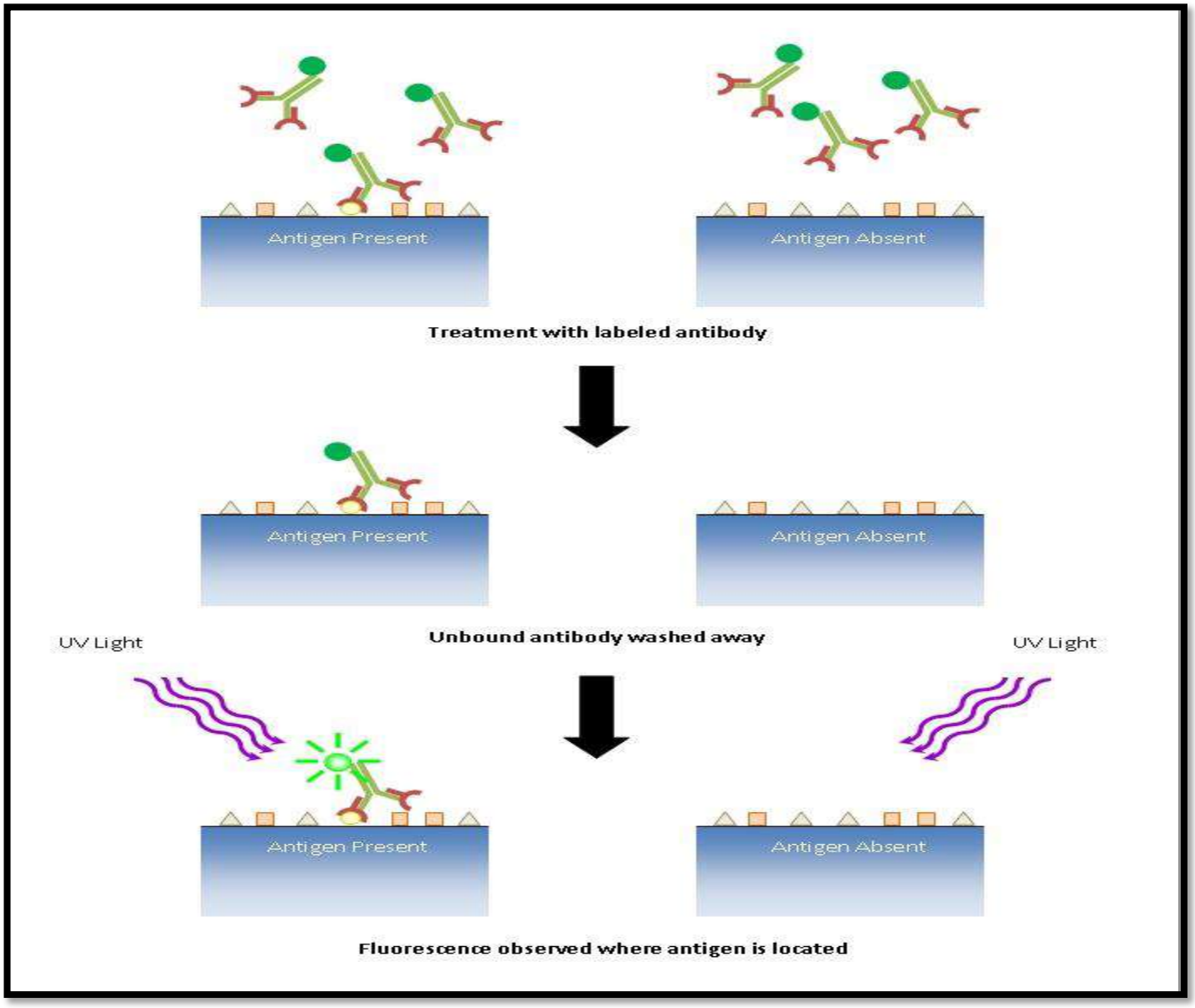
6- Slide is washed to remove unattached Ab's.

7- Examined under UV light in an fluorescent microscope.

8- The site where the Ab attaches to its specific Ag will show apple green fluorescence.

9- This technique can be used to detect pathogens or their Ag's in tissues or in pathological samples. viral, parasitic, tumor antigens from patient specimens or monolayer of cells.

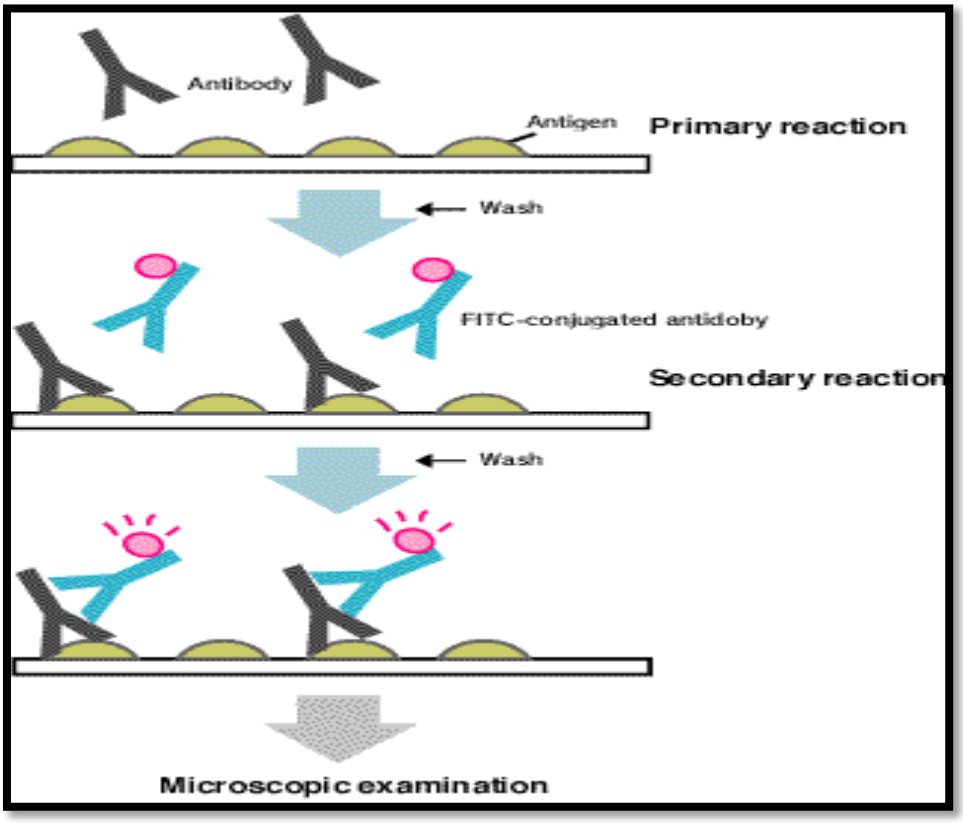
10- Another application is identification of anatomic distribution of an antigen within a tissue or within compartments.



Indirect immunofluorescence:

Is employed to detect antibodies in patient serum. The antigen on smear are made to react with specific unlabeled antibody and washed. The unbound antibody gets washed off adding another antibody. The second antibody is labeled anti-gamma globulin antibodies. This antibody binds to Fc portion of first antibody and persists despite washing. The presence of the second antibody is detecting by observing under fluorescent microscope.

It is often used to detect auto antibodies .Commonly used in the detection of anti-nuclear antibodies (ANA) found in the serum of patients with SLE. irIndect test is a double-layer technique. The unlabeled antibody is applied directly to the tissue substrate Treated with a fluorochrome-conjugated anti-immunoglobulin serum.



Microimmunofluorescence:

This is a serological technique employed to detect antibodies in patient serum. It works on the same principle as that of indirect immunofluorescence but is performed on Teflon slides with many wells dotted with antigens. This technique is used in the serodiagnosis of Q fever, Mediterranean spotted fever, Detection of IgG, IgA and IgM Antibodies to Chlamydia, toxoplasmosis, epidemic typhus etc.

الأسبوع الثالث عشر

الهدف التعليمي: التعرف على تفاعل سلسلة البلمرة.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Polymerase chain reaction (PCR)**

Polymerase chain reaction (PCR) is a common laboratory technique used to amplify or make millions of copies of a particular region of DNA. This DNA region can be anything the experimenter is interested in. For example, it might be a gene whose function a researcher wants to understand, or a genetic marker used by forensic scientists to match crime scene DNA with suspects. It was originally invented by Kary Mullis in 1985 and got the Nobel Prize in 1993.

Requirements:

i) A thermal cycler (an instrument having a microprocessor-controlled temperature cycling).
ii) DNA segment to be amplified.
iii) Two primers, which are oligonucleotides (about 10-18 nucleotides long), oriented with their ends facing each other so that DNA synthesis can occur between them.
iv) The enzyme Taq polymerase (a DNA polymerase) which is stable at high temperature.
v) MgCl2.
vi) dNTPs (deoxy nucleoside triphosphate: dATPs, dTTPs, dGTPs, dCTPs).

Procedure:

The DNA, from which a segment is to be amplified, is mixed with an excess of the two primer molecules, all the four kinds of dNTPs, MgCl2 and Taq polymerase in a reaction mixture. The DNA segment is amplified involving the following 3 steps:

i) Denaturation: The reaction mixture is heated to a high temperature (94-96ºC) so that the DNA molecule is denatured i.e. the two strands of DNA duplex get separated. Each strand of the target DNA then acts as a template for DNA synthesis.

ii) Annealing: The mixture is then cooled by lowering the temperature upto 55-65ºC. At this temperature, the two primers anneal to each of the single-stranded template DNA. Annealing occurs due to presence of complementary sequences located at the 3' ends of the template DNA.

iii) Extension: In this step, the temperature is so adjusted that the Taq polymerase becomes active. Synthesis of new DNA strand begins in between the primers, dNTPs and Mg2+. The optimum temperature for this polymerization is kept at 72ºC.

The next PCR amplification cycle begins as soon as all the stages of previous cycle end. During PCR operation, the extension product of one cycle serve as a template for subsequent cycles and each time the amount of DNA doubles. Thus, a single template molecule of DNA generates 2n molecules at the end of n cycles.

Types of PCR;

1- Conventional PCR: this is the most basic form of PCR, involving a series of temperature changes to amplify a specific DNA sequence.

2- Real-time PCR (qPCR): qPCR allows for the quantification of DNA or RNA molecules during the amplification process, often using fluorescent dyes or probes.

3- Reverse transcription PCR (RT-PCR): RT-PCR is used to analyze RNA by first converting it into complementary DNA (cDNA) using reverse transcriptase, and then amplifying the cDNA using PCR.

3- Multiplex PCR: this technique allows for the amplification of multiple DNA targets simultaneously in a single reaction by using multiple primer sets.

4- Nested PCR: is enhances the specificity of the reaction by using two sets of primers in two successive PCR reactions, where the second set of primers is nested within the product of the first reaction.

5- Hot start PCR: is a modification of PCR that improves specificity by preventing primer extension at lower temperatures, minimizing non-specific amplification.

6- Digital PCR: is a technique that partitions a PCR reaction into thousands of individual reactions, allowing for absolute quantification of target DNA molecules.

7- High-fidelity PCR: this type of PCR uses enzymes with proofreading activity to minimize errors during DNA amplification, resulting in a higher accuracy of the amplified product.

8- Long-range PCR: long-range PCR is designed to amplify long DNA fragments, typically longer than 10 kb.

9- In situ PCR: is performed directly on cells or tissue sections, allowing for the detection of DNA or RNA within their natural context.

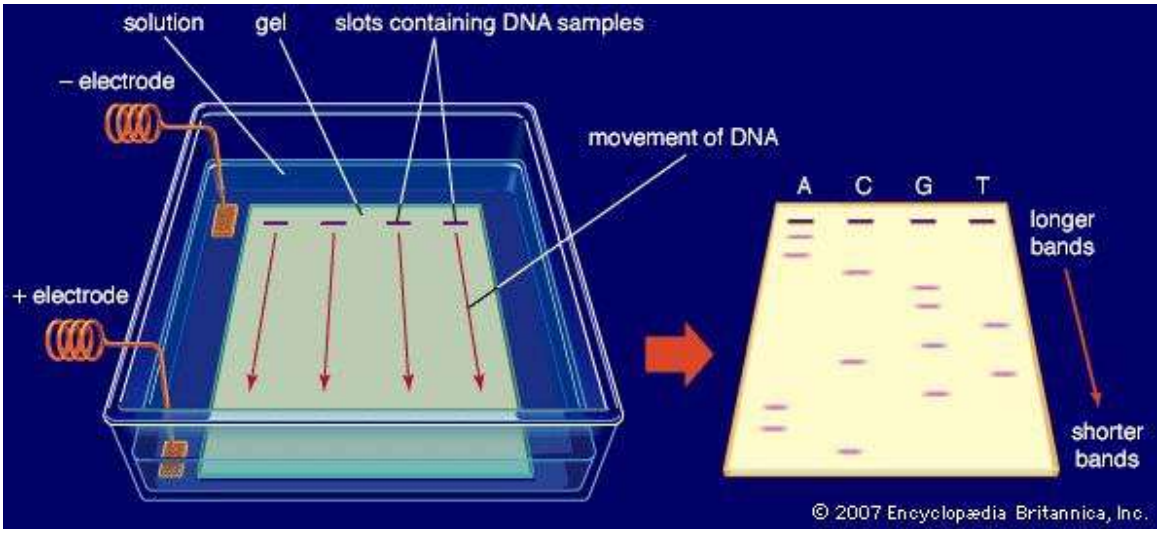
10- Asymmetric PCR: is amplifies only one strand of the target DNA, producing a large excess of one strand over the other.

11- Touchdown PCR: is a technique where the annealing temperature is gradually lowered during the PCR cycles, which improves the specificity of the reaction.

Gel Electrophoresis:

IT is a laboratory technique used to separate DNA, RNA, or protein molecules based on their size and electrical charge. It involves applying an electric field to a gel matrix, causing molecules to migrate through the gel at different rates depending on their size and charge.

Smaller molecules and molecules with a higher charge will migrate faster and further than larger, less charged molecules.



الأسبوع الرابع عشر

الهدف التعليمي: التعرف على تفاعل سلسلة البلمرة في الوقت الحقيقي.

مدة المحاضرة: 4 ساعات.

الأنشطة المستخدمة: أسئلة عصف ذهني.

أساليب التقويم: التغذية الراجعة النهائية (التقويم الختامي).

عنوان المحاضرة: **Real-Time (PCR)**

Real-Time PCR is a technique used to monitor the progress of a PCR reaction in real-time.

At the same time, a relatively small amount of PCR product (DNA, cDNA or RNA) can be quantified. Real-Time PCR is based on the detection of the fluorescence produced by a reporter molecule which increases, as the reaction proceeds. Real-Time PCR is also known as a quantitative polymerase chain reaction (qPCR), which is a laboratory technique of molecular biology based on the polymerase chain reaction (PCR).

qPCR is a powerful technique that allows exponential amplification of DNA sequences.

A PCR reaction needs a pair of primers that are complementary to the sequence of interest. Primers are extended by the DNA polymerase. The copies produced after the extension, so-called amplicons, are re-amplified with the same primers leading thus to exponential amplification of the DNA molecules. After amplification, however, gel electrophoresis is used to analyze the amplified PCR products and this makes conventional PCR time consuming; since the reaction must finish before proceeding with the post-PCR analysis. Real-Time PCR overcomes this problem.

The term “real-time” denotes that it can monitor the progress of the amplification when the process is going on in contrast to the conventional PCR method where analysis is possible only after the process is completed.

Procedure:

The working procedure can be divided into two steps:

a. Amplification:

1- Denaturation:

High temperature incubation is used to “melt” double- stranded DNA into single strands and loosen secondary structure in single-stranded DNA. The highest temperature that the DNA

polymerase can withstand is typically used (usually 95°C). The denaturation time can be increased if template GC content is high.

2- Annealing:

During annealing, complementary sequences have an opportunity to hybridize, so an appropriate temperature is used that is based on the calculated melting temperature (Tm) of the primers(5°C below the Tm of the primer).

3- Extension:

At 70-72°C, the activity of the DNA polymerase is optimal, and primer extension occurs at rates of up to 100 bases per second. When an amplicon in real-time PCR is small, this step is often combined with the annealing step using 60°C as the temperature.

b. Detection:

The detection is based on fluorescence technology. The specimen is first kept in proper well and subjected to thermal cycle like in the normal PCR. The machine, however, in the Real Time PCR is subjected to tungsten or halogen source that lead to fluoresce the marker added to the sample and the signal is amplified with the amplification of copy number of sample DNA. The emitted signal is detected by an detector and sent to computer after conversion into digital signal that is displayed on screen. The signal can be detected when it comes up the threshold level (lowest detection level of the detector).

Applications of Real-Time PCR:

- Gene expression analysis.
- Cancer research.
- Drug research.
- Disease diagnosis and management.
- Viral quantification.
- Food testing.
- GMO food.
- Animal and plant breeding.
- Gene copy number.